AD_____

AWARD NUMBER: W81XWH-06-1-0006

TITLE: Mechanism of Selenium Chemoprevention and Therapy in Prostate Cancer

PRINCIPAL INVESTIGATOR: Allen C. Gao, M.D., Ph.D.

CONTRACTING ORGANIZATION: University of California Davis
Sacramento, CA 95817

REPORT DATE: November 2009

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
1 November 2009	Annual	26 Sep 2008 – 30 Oct 2009

4. TITLE AND SUBTITLE

Mechanism of Selenium Chemoprevention and Therapy in Prostate Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-06-1-0006

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Allen C. Gao, M.D., Ph.D.

E-Mail: acgao@ucdavis.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of California Davis
Sacramento, CA 95817

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Prevention trials demonstrated that selenium is a promising chemopreventive agent for prostate cancer. Selenium inhibited human prostate cancer cell growth, blocked cell cycle progression, and induced apoptotic cell death. We have demonstrated a novel mechanism of selenium anticancer action in which selenium markedly reduces androgen receptor (AR) expression and AR-mediated gene expression including prostate-specific antigen (PSA) in human prostate cancer cells in vitro and in vivo. Based on our novel finding that selenium disrupts AR signaling by reducing AR expression, it is conceivable that selenium (reducing AR expression) might improve the efficacy of androgen deprivation therapy. In this application, we will test the effects of selenium on prostate cancer therapy.

15. SUBJECT TERMS
Selenium, therapy, prostate cancer

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	14	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Page

Introduction

The goal of this application is to elucidate the importance of down regulation of AR signaling by multiple selenium compounds and select the best leading selenium compound for prostate cancer chemoprevention and therapy. In this application, we will further study the mechanisms of AR downregulation by multiple selenium compounds and functional significance of this down regulation in prostate cancer chemoprevention and therapy. Prevention trials demonstrated that selenium reduced prostate cancer incidence by 50%, establishing selenium as a promising chemopreventive agent for prostate cancer. Selenium inhibited human prostate cancer cell growth, blocked cell cycle progression at multiple transition points, and induced apoptotic cell death. We have demonstrated a novel mechanism of selenium anticancer action in which selenium markedly reduces androgen receptor (AR) expression and AR-mediated gene expression including prostate-specific antigen (PSA) in human prostate cancer cells in vitro and in vivo. Androgen signaling through androgen receptor (AR) plays an important role not only in maintaining the function of the prostate, but also in promoting the development of androgen-independent prostate cancer. AR signaling is often hyperactive in androgen-independent prostate cancer. A common treatment for prostate cancer is androgen deprivation. Although most men respond to androgen deprivation therapy initially, almost all relapse due to the growth of androgen-independent cancer cells. Most of the androgen deprivation treatments are either blocking androgen-AR binding or reducing the levels of androgen. Based on our novel finding that selenium disrupts AR signaling by reducing AR expression, a completely different mechanism from the current androgen deprivation therapy, it is conceivable that targeting AR signaling by a combination of androgen-deprivation therapy and selenium (reducing AR expression) might improve the efficacy of current androgen deprivation therapy. This concept was validated in vitro in which the combination of selenium and anti-androgen (Casodex) synergistically inhibited clonogenic ability of human prostate cancer cells, providing a rationale for in vivo validation of the combination of selenium and anti-androgen therapy for prostate cancer. The hypothesis is that anticancer effects of multiple selenium compounds are mediated, in part, by inhibition of AR activity and that decreased AR signaling may reduce the incident of prostate cancer and prevent or delay relapses after androgen deprivation therapy. The goal of this application is to elucidate the importance of down regulation of AR signaling by multiple selenium compounds and determine the best leading selenium compound for prostate cancer chemoprevention and therapy.

Body

We have made significant progress of task 1 (i.e., To compare the effect of multiple selenium compounds and determine the molecular basis of the effects of multiple selenium compounds on AR expression (Months 1-8)**.**

MSA decreases AR mRNA stability Our results suggest that while MSA decreased AR mRNA levels at the transcriptional level, AR mRNA expression can also be regulated at post-transcriptional level. To examine whether MSA affects AR mRNA stability, LNCaP cells that express functional AR were treated with or without 5 μM of

MSA in the presence of actinomycin D (5 µg/ml) to stop de novo mRNA synthesis. The total RNA was isolated at different time points and AR mRNA levels were measured by Northern blot analysis. The half-life of AR mRNA was determined by comparison of mRNA levels over time between cells treated with or without actinomycin D, either in the presence or absence of MSA. Since actinomycin D is capable of inducing cell death, we monitored cell growth for a period of 24 h and did not observe cell death or growth inhibition with the concentration of actinomycin D used (5 µg/ml). We did not observe significant cell death or growth inhibition at 5 µM MSA over a period of 24 h in LNCaP cells. MSA treatment initially enhanced AR mRNA levels within 6 h. However, AR mRNA levels were significantly decreased by MSA compared to the control at 8 h. Figure 1 shows on the semi-log plot, the mean values of percentage of AR mRNA levels over time relative to respective time zero AR mRNA value as 100%. In MSA treated cells, AR half-life was reduced to about 7 h from 12 h in the control cells, suggesting that AR mRNA degradation was greatly accelerated in the presence of MSA after 6 h.

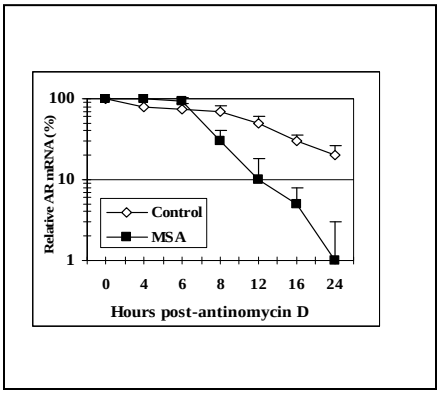
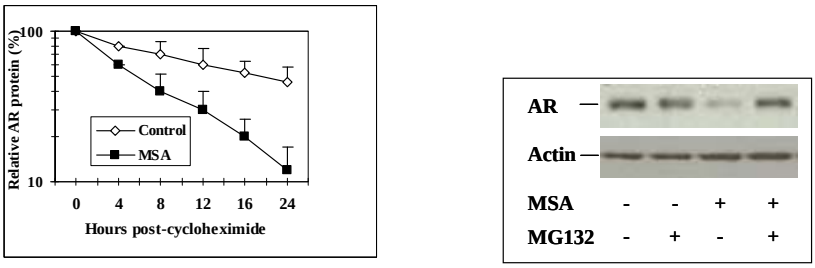


Figure 1 Effect of MSA on AR mRNA stability in LNCaP cells. The mRNA synthesis inhibitor antinomycin D (5 µg/ml) was added with or without 5 µM MSA at time 0. At specific time points, cells were harvested and total RNA as isolated by Northern blots. Points, means of three independent experiments plotted on semi-log scale relative to respective time zero AR mRNA value as 100%; bar, SD.

MSA increases AR protein turnover We have demonstrated that MSA decreased the levels of AR mRNA and protein in LNCaP cells. We next examined the effect of MSA on AR protein degradation after new protein synthesis was blocked by cycloheximide as a potential mechanism for downregulation of AR protein level. The protein synthesis inhibitor cycloheximide (50 µg/ml) was added with or without 5 µM MSA at time 0. At specified time points, cells were harvested and the levels of AR protein were measured by Western blot using anti-AR antibody. In MSA-treated cells, the half-life of AR protein was reduced to 6 h from 21 h in the control cells (Fig. 2A), suggesting that AR protein degradation was greatly enhanced in the presence of MSA. Systematic protein degradation by the ubiquitin-proteasome system plays an important role in the maintenance of protein stability. Protein ubiquitination provides the recognition signal for the 26S proteasome, leading to protein degradation. Studies demonstrated that AR protein level in cells is regulated by systemic protein degradation pathways. To examine whether selenium induced AR protein degradation via ubiquitin-proteasome system, the 26S proteasome inhibitor MG132 was added to the cells treated with MSA. MG132 was able to retard MSA effect on AR protein levels (Fig. 2B), suggesting that MSA induced AR degradation via a proteasome-dependent pathway.



A B

Figure 2. A. Effect of MSA on AR protein turnover in LNCaP cells. The protein synthesis inhibitor cycloheximide (50 μg/ml) was added with or without 5 μM MSA at time 0. At specific time points, cells were harvested and cell lysates were prepared. AR protein levels were determined by Western blot analysis using antibody specifically against AR and normalized to α-actin control. Points, means of three independent experiments plotted on semi-log scale relative to respective time zero AR value as 100%; bars, SD. **B.** Effect of MG132 on MSA induced AR protein degradation. MG132 (5 μM) was added to LNCaP cells together with cycloheximide (50 μg/ml) in the presence and absence of 5 μM of MSA. The cell lysates were prepared at 24 h. AR protein levels were determined by Western blot analysis using antibodies specifically against AR and α-actin as a control.

Selenium inhibits AR nuclear translocation AR typically translocates to the nucleus to exert its function on gene expression. To examine whether selenium affects the translocation of AR, Western blot analysis was performed using cell extracts from either cytosolic or nuclear extracts. LNCaP cells were cultured in charcoal stripped FBS for 3 days before adding 10 nM of DHT in the absence or presence of 10 μM MSA for 2 h. Nuclear and cytosolic fractions were prepared and used for Western blot analysis using the anti-AR antibody. DHT treatment increased the levels of AR protein expression in the nucleus which were reduced by the treatment with MSA (Fig. 3). In contrast, MSA had little effect on AR protein expression in the cytosol. The expression of RNA polymerase II (Pol II) and Hsp90 were used as markers for the integrity of the nuclear and cytosolic fractions, respectively. These results suggest that MSA suppresses AR signaling in part via interruption of AR nuclear translocation.

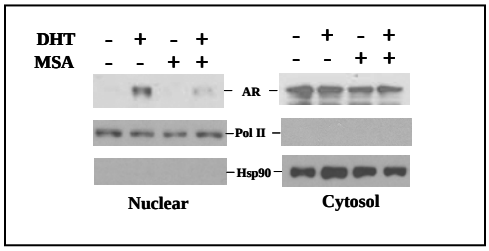


Figure 3. The effect of MSA on AR nuclear translocation. LNCaP cells were cultured in charcoal stripped FBS for 3 days and treated with 10 nM DHT with or without 10 μM MSA for 2 h. The cells were harvested for preparation of cytosolic and nuclear fractions and analyzed by Western blotting using antibodies against AR, Pol II, or Hsp90. The expression of Pol II and Hsp90 were used as markers for the integrity of the nuclear and cytosolic fractions, respectively.

Selenium inhibits the recruitment of coactivators and enhances the recruitment of corepressors to AR target genes AR interacts with coregulators to achieve maximal transactivation activity. To examine the effects of selenium on the recruitment of coregulators to the promoters of AR target genes, chromatin immunoprecipitation (ChIP) analysis was performed. DHT increased the recruitment of AR and TIF-2, SRC-1 to the promoter of PSA gene in the absence of MSA and this recruitment was greatly diminished in the presence of 5 μM MSA (Fig. 4). On the other hand, MSA treatment prevented the nuclear translocation of AR in the presence of hormone, thus the corepressors including SMRT and NCoR remain bound to the promoter of the PSA gene (Fig. 4). These results suggest that MSA-mediated reduction of AR activation may be due, at least in part, to a decrease in the recruitment of AR and its coactivators to the promoter of the AR target gene PSA, while maintains corepressors bound to the promoter.

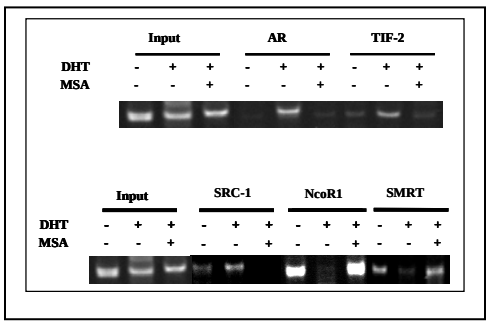


Figure 4. Effect of MSA on the recruitment of AR and coregulators to the promoter of an endogenous AR target gene, PSA. The *in vivo* binding of AR and coregulators to the PSA promoter was examined by the ChIP assay. LNCaP cells were cultured in charcoal stripped condition for 3 days. Soluble chromatin was prepared from cells treated with 10 nM DHT for 4 h (+) or untreated (-) in the presence (+) or absence (-) of 10 μM MSA and immunoprecipitated with antibodies against AR, TIF-2, SRC-1, SMRT, and NCoRI. Co-precipitated DNA was amplified by PCR using primers that flank the ARE in the PSA promoter region. The presence of total PSA promoter DNA in the soluble chromatin prior to immunoprecipitation was included as input.

We have made progression on task 2 (To determine the role of AR in selenium growth inhibition in prostate cancer).

We have demonstrated that overexpression of AR interferes with MSA-mediated growth inhibition. In an effort to evaluate the biological significance of MSA suppression of androgen receptor signaling, we transiently transfected LNCaP cells with a wild-type androgen receptor and assessed the response of the androgen receptor–overexpressing cells to MSA-induced growth inhibition. The MTT assay was conducted at 48 hours post-MSA, and the data are presented in Fig. 5A. In the absence of MSA, cell growth was not altered by the transfection of androgen receptor (data not shown), indicating that the endogenous level of androgen receptor is not a limiting factor for the growth of these cells. MSA treatment inhibited growth by 40% in the mock transfectants, as opposed to 27% in the androgen receptor transfectants. The difference is statistically significant (P =

0.003). Thus, androgen receptor overexpression was able to weaken the growth suppressive activity of MSA. One reason that the difference was seemingly compressed was due to the fact that only a fraction of cells was successfully transfected, and in this study, cell growth was assessed using the whole cell population. To address the last problem, we cotransfected cells with the androgen receptor expression vector and a membrane-GFP-encoding construct. The cells were then subjected to BrdUrd labeling, and the data were analyzed by gating just the GFP-positive cells. As shown in Fig. 5B, after selecting for the subset of GFP-positive cells, we found that MSA inhibited DNA synthesis by a very modest 16% in the androgen receptor transfectants, as opposed to 72% in the mock transfectants. Because the GFP and androgen receptor cDNAs are not located in the same plasmid construct, it is possible that not all the cells positive for GFP are also positive for the transfected androgen receptor. Thus, our selection process only led to an enrichment, rather than an exclusive selection, of double-positive cells. Therefore, the difference between the mock transfectants and the androgen receptor transfectants might have been even more pronounced if all the cells used in the experiment were successfully transfected with androgen receptor. Figure 5B also shows that when we did the BrdUrd labeling experiment with the nonenriched androgen receptor–transfected cells, the inhibition by MSA was about 45%, a value half-way between that achieved by the mock transfectants and the enriched androgen receptor transfectants.

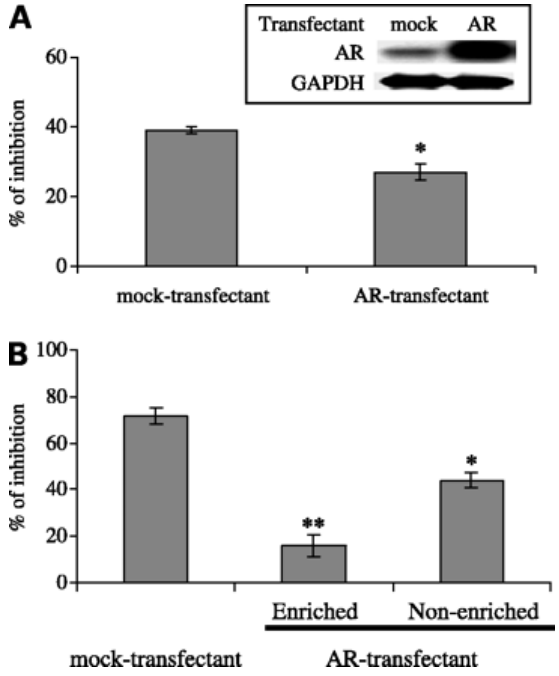


Figure 5. Effect of androgen receptor (*AR*) overexpression on MSA inhibition of cell growth. **A,** MTT cell growth assay in androgen receptor– or mock-transfected LNCaP cells treated with MSA. Western blot confirmation of androgen receptor protein level (*inset*). **B,** BrdUrd labeling of selected GFP-positive or nonselected androgen receptor–transfected LNCaP cells treated with MSA. *Columns*, % inhibition compared with untreated control. *, $P < 0.05$, statistically different from mock transfectant. **, $P < 0.05$, statistically different from mock transfectant and nonselected androgen receptor transfectant.

We have made progress on task 3 (*To evaluate combination of selenium and anti-androgen therapies in mouse models of prostate carcinogenesis***).**

Combination of selenium and anti-androgen synergistically reduces AR transactivation

A common treatment modality for prostate cancer is androgen deprivation. The goal of these androgen deprivation treatments is either blocking androgen-AR binding or reducing the levels of androgen. Although anti-androgen treatment is effective, the anti-tumor effects can be temporary. Numerous studies have demonstrated that AR is expressed and AR signaling remains intact and is often hyperactive in androgen-independent prostate cancer. Selenium decreases AR expression and reduces AR activation provides a molecular basis for selenium chemoprevention and chemotherapy targeting AR signaling in prostate cancer. We hypothesize that an intervention strategy aimed at both blocking ligand binding (chemical or surgical castration) and dampening AR expression (selenium treatment) would be achieve better therapeutic effect than either alone. To test this hypothesis, we first examined whether combination of selenium with anti-androgen, flutamide (Flu), can synergistically inhibit AR transactivation in human prostate cancer cells *in vitro*. C4-2 cells were transfected with ARE-containing luciferase reporter and treated with either flutamide or MSA alone, or the combination of flutamide and MSA. The ARE luciferase activity was reduced by either flutamide or MSA alone, however, combination of flutamide and MSA achieved much greater inhibition of ARE luciferase activity than either flutamide or MSA alone (**Fig. 6**), suggesting that blocking AR ligand binding and reducing AR expression may synergistically inhibit AR transactivation in prostate cancer cells.

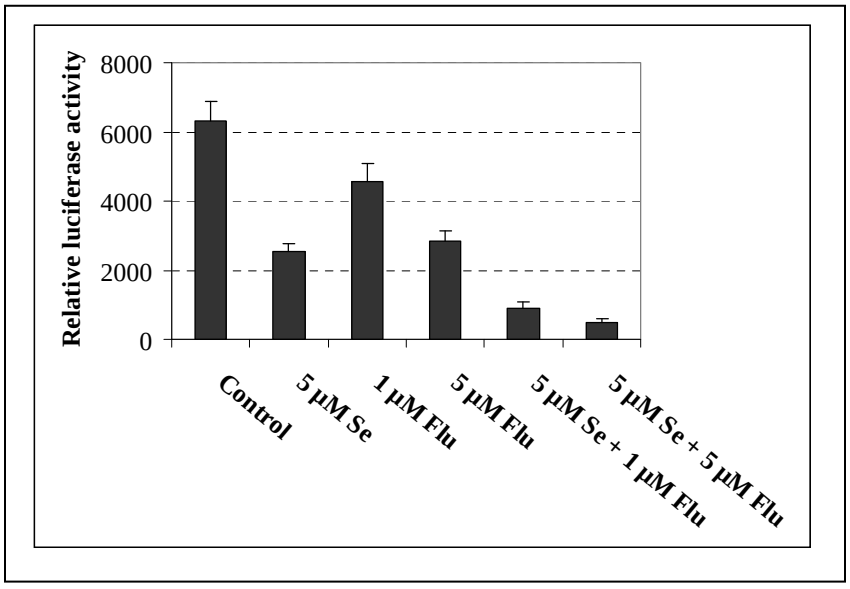


Fig. 6. The effect of combination of selenium (Se) and flutamide (Flu) on AR activation in C4-2 cells. C4-2 cells were transfected with plasmid containing androgen responsive element (ARE)-luc and treated with either Se or Flu alone or together as indicated. Luciferase activity was determined and normalized to the amount of protein.

The effects of combination of MSA and antiandrogen agents on clonogenic ability *in vitro* in C4-2 cells To test whether reduction of AR transactivation by the combination of flutamide with MSA affects cell clonogenic ability *in vitro*, C4-2 cells were treated with either flutamide or MSA alone or together, and clonogenic ability was determined. Treatment with flutamide or MSA alone reduced C4-2 clonogenic ability, combination of flutamide and MSA inhibited C4-2 clonogenic ability much greater than either one alone (**Fig. 7**), suggesting that combination of flutamide with MSA can synergistically inhibit C4-2 cell clonogenic ability *in vitro*.

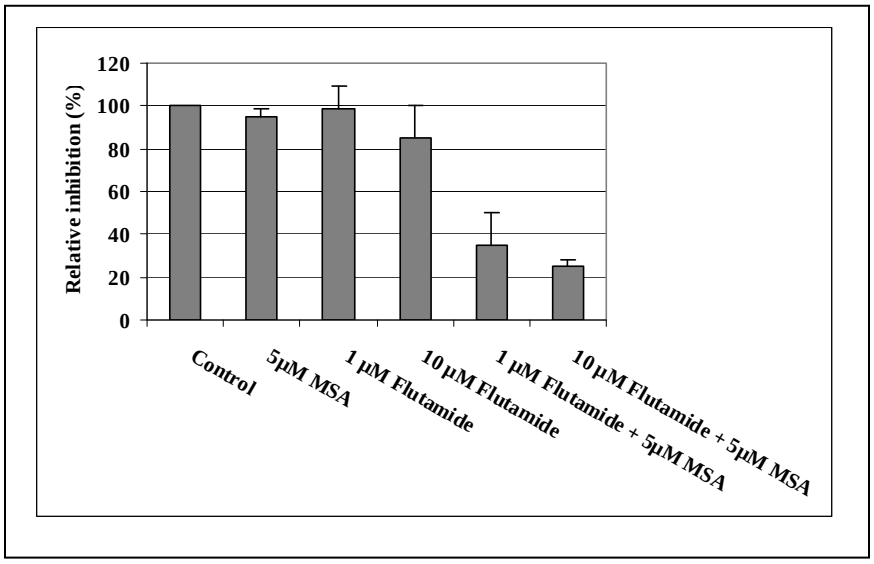


Fig. 7. The effects of combination of selenium (Se) and flutamide (Flu) treatment on clonogenic ability of C4-2 cells.

Effect of combination of selenium and anti-androgen on xenograft models of human prostate cancer

Having demonstrated that combination of selenium and anti-androgen agents can achieve better efficacy on inhibiting AR activation and cell clonogenic ability, we next test the effects of the combination on in vivo tumor growth. The effect of combination of methylselenocysteine (MSC) and hydroxyflutamide on tumor growth of C4-2 prostate cancer growth was demonstrated *in vivo*. The male nude mice were co-inoculated with 3×10^6 cells and Matrigel. When the tumors became palpable, the mice were divided into 4 groups with 8 mice in each group. One group was treated with 100 μg MSC, the rest groups were treated with 500 mcg of hydroxyflutamide, 500 mcg hydroxyflutamide plus 100 μg MSC, and vehicle control by i.p. injection daily for 12 days, respectively. Tumor volume was measured 3 times a week. As shown in **Fig. 8**, MSC + flutamide had a greater effect on suppression of tumor growth than flutamide alone.

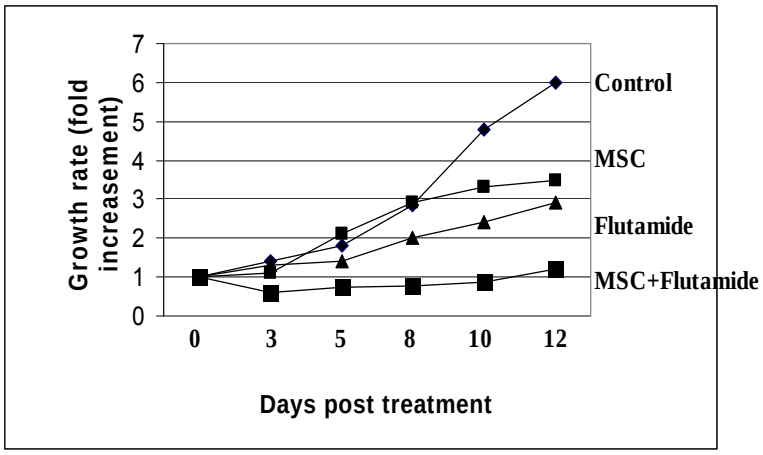


Figure 8. The effect of combination of MSC and hydroxyflutamide on tumor growth of C4-2 prostate cancer growth *in vivo*. The male nude mice were co-inoculated with 3 x 10^6 cells and Matrigel. When the tumor became palpable, the mice were divided into 4 groups with 8 mice in each group. One group was treated with 100 mcg MSC, the rest groups were treated with 500 mcg of hydroxyflutamide, 500 mcg hydroxyflutamide plus 100 mcg MSC, and vehicle control by i.p. injection daily for 12 days, respectively. Tumor volume was measured 3 times a week.

Effect of combination of MSC and flutamide treatment on AR, Akt expression

We previously demonstrated that selenium significantly suppressed AR expression and AR-regulated gene PSA expression in LNCaP cells *in vitro*. To determine whether selenium affects AR expression *in vivo*, we determined AR protein expression in the C4-2 bearing tumor tissues using Western blot. The levels of AR protein expression were considerable decreased by the treatment with either MSC or flutamide alone and by the combination of MSC and flutamide (**Fig. 9**). The expression of phosphorylate Akt was also decreased by the treatment with either MSC or flutamide alone, and the expression was further decreased by the combination of MSC and flutamide (**Fig. 9**).

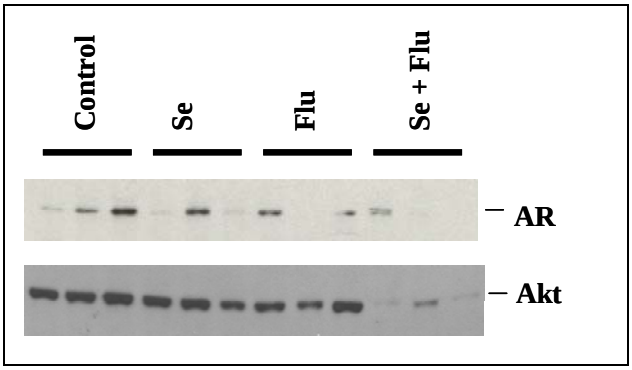


Fig. 9. AR and Akt protein expression in tumors by Western blot analysis. Nuclear extracts were isolated from tumors of individual mice and indicated and subjected to Western blot analysis.

We recently found that selenium prevent SP1 protein binding to AR promoter, which could potentially decrease AR mRNA transcription.

We also found that selenium down regulate Hsp90 protein expression. Since Hsp90 is a AR chaperone protein, it is possible that selenium down-regulates AR by modulating Hsp90 chaperone function. We are currently performing experiments to further understanding the effects of selenium on Hsp90 and AR regulation in prostate cancer cells.

Key research accomplishments

- We demonstrated that selenium downregulates AR signaling in prostate cancer cells.
- MSA decreases AR mRNA stability.
- MSA increases AR protein turnover.
- Selenium inhibits AR nuclear translocation.
- Selenium inhibits the recruitment of coactivators and enhances the recruitment of corepressors to AR target genes.
- Combination of selenium with antiandrogen significantly reduced the number of colony formation than either selenium or antiandrogen alone.
- Combination of selenium with antiandrogen has a greater effect on suppression of tumor growth than either flutamide or selenium alone.
- Combination of selenium with antiandrogen significantly reduced the levels of AR and Akt protein expression in tumors.
- Selenium prevents SP1 protein binding to AR promoter, which could potentially decrease AR mRNA transcription.
- Selenium down regulates Hsp90 protein expression. Since Hsp90 is a AR chaperone protein, it is possible that selenium down-regulates AR by modulating Hsp90 chaperone function.

Reportable outcome

Publications:

1. Dong Y, Lee SO, Zhang H, Marshall J, Gao AC and Ip C. Prostate specific antigen (PSA) expression is down-regulated by selenium through disruption of androgen receptor signaling. Cancer Res. 64 (1): 19-22, 2004, (Gao, AC, corresponding author).
2. Lee SO, Nadiminty N, Wu XX, Lou W, Dong Y, Ip C, Onate SA, and Gao AC. Selenium disrupts estrogen signaling by altering estrogen receptor expression and ligand binding in human breast cancer cells. Cancer Res 65(8):3487-3492, 2005.
3. Dong Y, Zhang HT, Gao AC, Marshall JR, and Ip C. Androgen receptor signaling intensity is a key factor in determining the sensitivity of prostate cancer cells to selenium inhibition of growth and cancer-specific biomarkers. *Molecular Cancer Therapeutics* 4: 1047-1055, 2005.

4. Lee SO, Chun JY, Nadiminty N, Trump DL, Ip C, Dong Y, and Gao AC. Monomethylated selenium inhibits growth of LNCaP human prostate cancer xenograft accompanied by a decrease in the expression of androgen receptor and prostate-specific antigen (PSA). *Prostate, 66: 1070-1075,* 2006.
5. Zhang H, Dong Y, Zhao HJ, Brooks JD, Hawthorn L, Nowak N, Marshall JR, Gao AC, and Ip C. Microarray data mining for potential selenium targets in chemoprevention of prostate cancer . Cancer Genomics & Proteomics 2: 97-114, 2005.
6. Chun JY, Nadiminty N, Lee SO, Onate SA, Lou W, and Gao AC. Mechanisms of selenium down-regulation of androgen receptor signaling in prostate cancer. Molecular Cancer Therapeutics 5 (4): 913-918, 2006.
7. Chun JY, Hu Y, Pinder E, Wu JG, Li F, and Gao AC. Selenium inhibition of survivin expression by preventing Sp1 binding to its promoter. *Molecular Cancer Therapeutics 6(9):2572-80, 2007*
8. Nadiminty N and Gao AC. Mechanisms of selenium chemopreventio n and therapy in prostate cancer. Mol Nutr Food Res, Aug 22, 2008.
9. Nadiminty N and Gao , AC. Selenium and androgen receptor signaling in prostate cancer. Androgen Action in Prostate Cancer. Edited by Tindall D and Mohler J. Springer Science and Business Media and Humana Press, 2009

Abstract:

1. Lee SO, Chun JY, Nadiminty, N, and Gao AC. Selenium inhibits growth of LNCaP human prostate tumor accompanied by a decrease in the expression of androgen receptor and prostate-specific antigen (PSA). Innovative Minds in Prostate Cancer Today (IMPaCT) meeting. Proceedings P18-14, p206, Atlanta, GA, September 5-8, 2007.

Conclusions

- We demonstrated that selenium downregulates AR signaling via multiple pathways including decreases AR mRNA and protein expression, decreases AR mRNA stability, increases AR protein turnover, inhibits AR nuclear translocation, and affects the recruitment of coregulators to the androgen responsive genes.

- Combination of selenium and anti-androgen therapies has better antitumor effect than either selenium or anti-androgen alone.

References

1. Dong Y, Zhang HT, Gao AC, Marshall JR, and Ip C. Androgen receptor signaling intensity is a key factor in determining the sensitivity of prostate cancer cells to

selenium inhibition of growth and cancer-specific biomarkers. *Molecular Cancer Therapeutics* 4: 1047-1055, 2005.

2. Dong Y, Lee SO, Zhang H, Marshall J, Gao AC and Ip C. Prostate specific antigen (PSA) expression is down-regulated by selenium through disruption of androgen receptor signaling. *Cancer Res*. 64 (1): 19-22, 2004.
3. Chun JY, Nadiminty N, Lee SO, Onate SA, Lou W, and Gao AC. Mechanisms of selenium down regulation of androgen receptor signaling in prostate cancer. *Molecular Cancer Therapeutics*, 5 (4): 913-918, 2006.
4. Lee SO, Chun JY, Nadiminty N, Trump DL, Ip C, Dong Y, and Gao AC. Monomethylated selenium inhibits growth of LNCaP human prostate cancer xenograft accompanied by a decrease in the expression of androgen receptor and prostate-specific antigen (PSA). *Prostate 66: 1070-1075,* 2006.